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Distribution of plasma folate forms in hemodialysis patients receiving high daily doses of L-folinic or folic acid

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Background. We have previously reported that a daily oral high dose of L-folinic acid for the treatment of hyperhomocysteinemia in hemodialysis patients does not provide significantly greater reduction in fasting total homocysteine (tHcy) levels than an equimolar dose of folic acid. The present study uses the affinity/HPLC method to analyze the distribution of plasma folate forms in patients who received L-folinic acid versus those who received folic acid. This was done to investigate claims that renal insufficiency is associated with impaired folate interconversion, a stance that is supportive of the premise that tHcy lowering in these patients is more efficacious with folinic acid and other reduced folates, than folic acid.

Methods. Forty-eight chronic and stable hemodialysis patients were block-randomized, based on their screening predialysis tHcy levels, sex, and dialysis center, into two groups treated for 12 weeks with oral folic acid at 15 mg/day or an equimolar amount (20 mg/day) of oral L-folinic acid. All 48 subjects also received 50 mg/day of oral vitamin B_6 and 1 mg/day of oral vitamin B_{12}. Folate distribution was determined in plasma of 46 participants (Folinic acid group, $N = 22$; Folic acid group, $N = 24$) by using the affinity/HPLC method, with electrochemical (coulometric) detection.

Results. Both groups had similar baseline geometric means of plasma total folate and similar folate forms distribution. Following treatment, both groups demonstrated similar marked elevation in plasma total folate (geometric mean of the increase: Folinic acid group, +337 ng/mL; Folic acid group, +312 ng/mL; $P = 0.796$). In the folinic acid-treated group, practically all of the increase in total folate was due to 5-methyltetrahydrofolate. In the folic acid-treated group 5-methyltetrahydrofolate accounted for 35% of the increase in total folate and the remainder was unmethylated folic acid.

Conclusions. Data from the present findings suggest that defects in folate absorption or impairment in folate interconversion are not the cause of the persistent hyperhomocysteinemia in hemodialysis patients.

Key words: end-stage renal disease, homocysteine, maintenance hemodialysis, hyperhomocysteinemia, 5-methyltetrahydrofolate, pteroyglutamic acid.

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Elevation of plasma total homocysteine (tHcy) is observed in at least 85% of patients with end-stage renal disease (ESRD) undergoing maintenance hemodialysis [1]. Several attempts have been made to lower the plasma tHcy levels in hemodialysis patients. For example, our previous study showed that a final treatment value of plasma tHcy <12 μmol/L was achieved in only one of 15 (6.7%) dialysis patients despite two months of supplementation with a total of 16 mg per day of folic (pteroyglutamic) acid, 50 mg per day of vitamin B_6, and 1 mg per day of vitamin B_{12} [2].

In an open-label, uncontrolled investigation, Touam et al concluded that high doses of the reduced folate L–5-formyltetrahydrofolate (L-folinic acid) might provide improved tHcy-lowering efficacy in patients with ESRD undergoing maintenance hemodialysis [3]. We recently reported results from a randomized controlled trial comparing the tHcy-lowering efficacy of equimolar amount of L-5-methytetrahydofolate (17 mg/day) versus folic acid (15 mg/day)-based B vitamin supplementation in 50 (that is, two matched groups of 25) hemodialysis patients. After 12 weeks of treatment, the mean percentage reductions in predialysis tHcy were not significantly different (L-5-methytetrahydofolate group, 17%; Folic acid group, 14.8%; $P = 0.444$) [4]. We also conducted a block randomized, controlled comparison of oral treatment with either high-dose L-folinic acid or folic acid, combined with oral vitamin B_6 and vitamin B_{12}, in 48 (that is, two matched groups of 24) maintenance hemodialysis patients. Relative to the high dose of folic acid, high-dose oral L-folinic acid–based B vitamin supplementation did not improve the tHcy-lowering efficacy in hemodialysis patients. Final post-treatment tHcy mean values (with 95% CI) were: Folinic acid group, 15.9 μmol/L (22.1% decrease); Folic acid group, 16.9 μmol/L (20.7% decrease; $P = 0.950$) [5]. The present study used our newly developed affinity/high-pressure liquid chromatography (HPLC) method [6] to analyze plasma folate forms distribution in hemodialysis patients receiving higher doses

of L-folinic acid versus those receiving folic acid. Our goal in the current study was to assess, as suggested by certain investigations [7–11], whether the metabolism of supplemented folic acid to 5-methyltetrahydrofolate is impaired in hemodialysis patients.

METHODS

Plasma was analyzed from 46 hemodialysis patients who participated in a study described previously [5]. Briefly, participants comprised 48 chronic (that is, hemodialysis duration ≥6 months) stable hemodialysis paients who were prescribed a daily multivitamin that contained 1.0 mg folic acid, 10.0 mg vitamin B_6, and 0.012 mg vitamin B_{12}. This baseline supplementation regimen was continued throughout the 12-week investigation. Participants were randomly assigned in paired blocks to one of two treatment regimens: (*1*) Folic acid group ($N = 24$) prescribed folic acid 15.0 mg/day, vitamin B_6 50.0 mg/day, and vitamin B_{12} 1.0 mg/day; and (*2*) Folinic acid group ($N = 24$) prescribed L-5-formyltetrahydrofolate 20.0 mg/day (that is, equimolar to 15.0 mg/day folic acid; Eprova, Zurich, Switzerland); vitamin B_6 50.0 mg/day; and vitamin B_{12}, 1.0 mg/day. Nonfasting, prehemodialysis blood samples were collected twice before treatment and twice during the 12th week of treatment, as described elsewhere [5]. All reported values are based on the averages of two pretreatment and post-treatment values. Laboratory analyses, data entry and data analyses were performed by code so that treatment assignments remained concealed.

Plasma folate analysis

The plasma folate forms distribution of 46 participants (Folinic acid group, $N = 22$; Folic acid group, $N = 24$) was determined using our newly devised affinity/HPLC method with electrochemical (coulometric) detection [6]. Plasma was diluted tenfold with extraction buffer (0.05 mol/L potassium tetraborate, 1% sodium ascorbate, pH 9.2), heat extracted (100°C for 15 min) and centrifuged for 15 minutes at $36{,}000 \times g$. Two milliliters of the supernatant fraction was injected onto the affinity column (10×4.6 mm) that contained purified milk folate binding protein covalently bound to AffiPrep 10 support (Bio-Rad, Richmond, CA, USA). After washing the affinity column sequentially with 0.05 mol/L potassium phosphate, pH 7, and water the folates were eluted onto the analytical column (Betasil Phenyl, 250 × 4.6 mm; Keystone Scientific, Bellefonte, PA, USA) with an acid mobile phase (0.028 mol/L dipotassium phosphate and 0.06 mol/L phosphoric acid in water). Folates then were eluted from the analytical column by using the same aqueous mobile phase at a flow rate of 1 mL/min for six minutes followed by a linear gradient over 50 minutes to the same mobile phase containing 20% acetonitrile (vol/vol). This elution separates folates both on the basis of their pteridine ring structure and number of glutamate residues. Folate forms elute in the following order: tetrahydrofolate (THF); 5-methylTHF; 5,10-methyleneTHF, 5- and 10-formylTHF; dihydrofolate; and pteroyglutamate. Folate activity is determined by using an ESA Four Channel Coularray Detector (ESA, Chelmsford, MA, USA) with channels 1 to 4 set at 0, 300, 500 and 600 mV, respectively. Quantification and identification of individual folates was done by comparison to external folate standards of known concentration. The coefficient of variations (CVs) for folate detection (4 to 30 ng/mL) by the described method ranged between 0.6 and 6.4% (intra-assay) and 5.2 and 8.4% (interassay). This variation included the duplicate samples taken before and after treatment.

Statistical analysis

All values reported are based on average of two pretreatment and post-treatment values. Baseline continuous variables were compared by paired *t* tests, and categorical variables were compared by chi square analysis. Continuous variables were assessed using both untransformed and (natural log) transformed values. Reported *P* values were based on two tailed calculations. Differences were considered to be significant if $P < 0.05$. All statistical analyses were performed using SYSTAT software (version 10.0; SPSS, Chicago, IL, USA).

RESULTS

The block randomization of this study was successful with respect to the key baseline covariables [5]. Plasma folate distribution analyses revealed that both 5-methyltetrahydrofolate and pteroyglutamate were the main folate forms present in plasma of all patients. Figure 1 represents typical chromatograms of folate distribution before and after treatment for each group. Analysis of baseline plasma folate distribution in the 46 patients is summarized according to treatment group in Table 1. Both groups had a similar baseline geometric mean of total plasma folate (Folinic acid group, 43.2 ± 0.4 ng/mL; Folic acid group, 31.1 ± 0.5 ng/mL; $P = 0.207$), and similar folate forms distribution (5-methyltetrahydrofolate: Folinic acid group, 33.1 ± 0.4 ng/mL, Folic acid group, 26 ± 0.4 ng/mL, $P = 0.295$; Folic acid: Folinic acid group, 5 ± 0.8 ng/mL, Folic acid group, 4.7 ± 0.8 ng/mL, $P = 0.908$). Following treatment both groups demonstrated a similar marked elevation in plasma total folate (geometric mean of the increase: Folinic acid group, +337 ng/mL; Folic acid group, +312 ng/mL; $P = 0.796$; Table 2). In the folinic acid group 96% of this increase was accounted by 5-methyltetrahydrofolate. In the folic acid group 35% of the increase in total folate was due to 5-methyltetrahydrofolate, the rest being unmethylated folic acid.

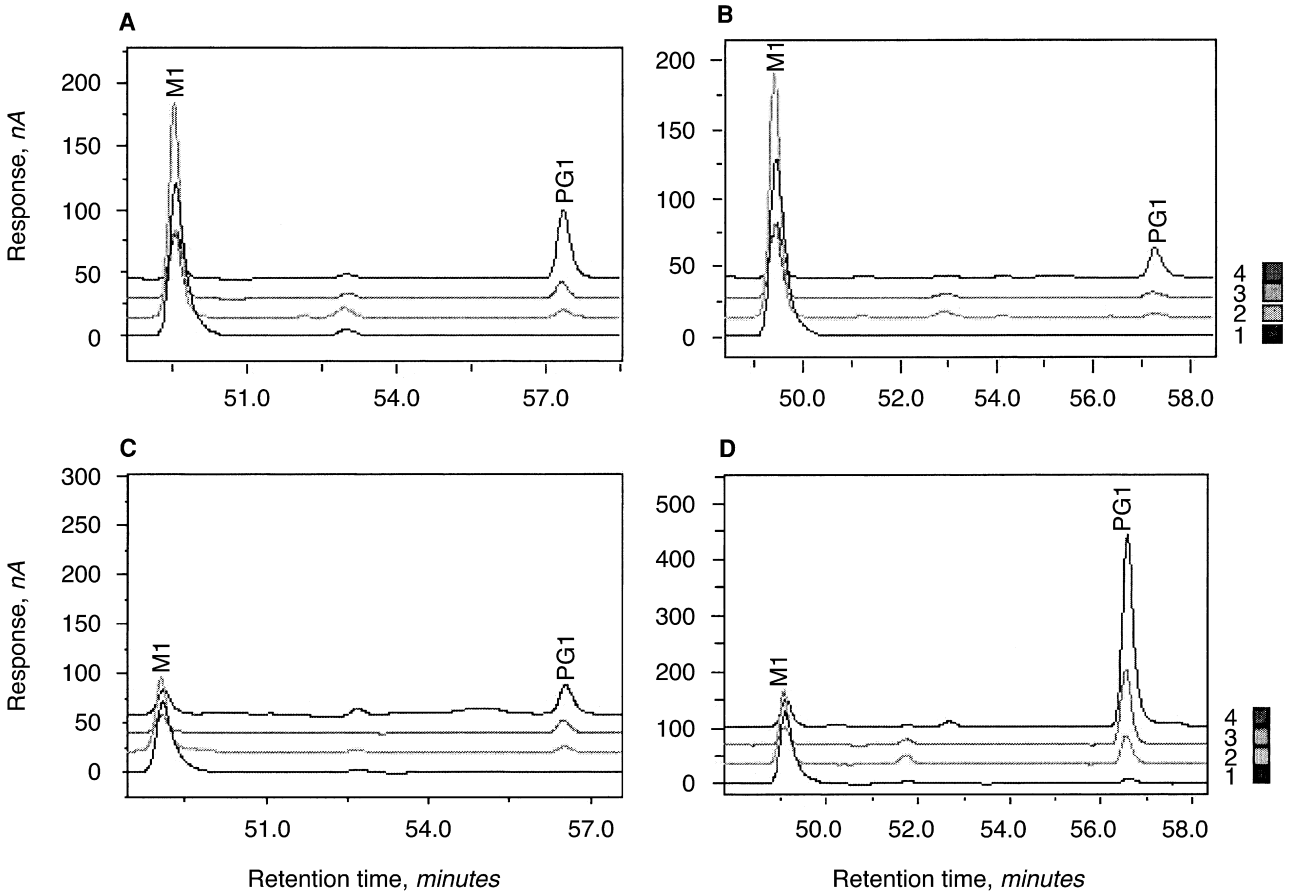

Fig. 1. Representative chromatograms of folate forms distribution. All panels show the electrochemical response of channels 1, 2, 3 and 4 set at 0, 300, 500 and 600 mV, respectively. Panels (*A*) and (*B*) represent plasma folate distribution for the folinic acid group before and after treatment, respectively. Panels (*C*) and (*D*) represent plasma folate distribution for the folic acid group before and after treatment, respectively. In both groups, plasma samples obtained after treatment (B and D) were diluted 1:5 with 1% sodium ascorbate before HPLC analysis. Abbreviations are: M, 5-methyltetrahydrofolate; PG, folic acid (pteroyglutamate); The number after the abbreviation indicates the number of glutamate residues.

Table 1. Baseline folate forms distribution by treatment group

Treatment group	Total folates *ng/mL*	Folate distribution *% of total folates* MTHF[a]	PteGlu[a]
Folic acid	31.1 ± 0.5 (20.7–46.7)	83.6%	15.1%
Folinic acid	43.2 ± 0.4 (30.6–60.8)	76.6%	11.5%

Values are geometric means ± SE (with 95% CI) for total folate, and % of 5-methyltetrahydrofolate (MTHF) and pteroyglutamate (PteGlu) from total folate.

[a] Differences are not significant between the two groups

Table 2. Treatment effects on folate forms distribution

Treatment group	Total folates *ng/mL*	Folate distribution *% of the increase* MTHF	PteGlu
Folic acid	+312.6 (160–613)	35%[a]	70%[a]
Folinic acid	+337.8 (214–532)	96%	2%

Values are geometric means of the increase (with 95% CI) for total folate, and % of MTHF and PteGlu from the increase of total folate. Abbreviations are: MTHF, 5-methyltetrahydrofolate; PteGlu, pteroyglutamate. Treatment groups were: (1) Folic acid, 15 mg folic acid, 50 mg vitamin B_6, and 1.0 mg vitamin B_{12}; (2) Folinic acid, 20 mg L-folinic acid, 50 mg vitamin B_6, and 1.0 mg vitamin B_{12}.

[a] $P < 0.05$ compared to folinic acid group within each folate form

DISCUSSION

The homocysteine lowering capacity of supplemental folic acid lies in the propensity of enzymes within the intestine and other peripheral tissues to catalyze the conversion of this vitamin to folate coenzymes. This includes reduction to tetrahydrofolate and subsequent one carbon substitution, either at the formate (formyltetrahydrofolate) or at the formaldehyde (methylene-tetrahydrofolate) levels of oxidation. These are then reduced to 5-methyltetrahydrofolate, the substrate for homocysteine methylation to methionine [12]. The present study shows that daily supplementation with high doses of folic acid or folinic acid for 12 weeks resulted in two substantial changes in plasma folate. First, there was an almost eight- to tenfold increase in the levels of total folate in plasma in either group. This large increase in total folate refutes the notion that hemodialysis patients have impaired intestinal folate absorption [7–11].

Second, a substantial proportion of this rise in plasma folate was comprised of 5-methyltetrahydrofolate. This high level of 5-methyltetrahydrofolate strongly implies that in the process of absorption and consequent transport in the liver and other peripheral tissues, folic acid and folinic acid underwent enzymatic conversion to this form of folate. This is consistent with earlier studies [13, 14], and supported by recent studies from our laboratory showing that the intestine is highly efficient in the conversion of folinic acid and other reduced folates to 5-methyltetrahydrofolate [4, 5]. The fact that in the folic acid group only 35% of plasma folate is comprised of 5-methyltetrahydrofolate is consistent with the study cited earlier, which showed that the conversion of folic acid to folate coenzymes is substantially slower because of the reduction step that is rate limiting.

Nevertheless, even 35% conversion is potentially sufficient for an effective homocysteine methylation. It amounts to 110.8 ng methyltetrahydrofolate per mL plasma, an amount that is tenfold higher than found in normal healthy individuals. These data refute any notion that in hemodialysis patients the conversion of folic acid to 5-methyltetrahydrofolate is impaired [7–11]. Furthermore, in contrast to one view [10] there is no evidence for the presence of folate in plasma in other than monoglutamyl derivatives, among either subjects with normal renal function, or patients with chronic renal disease.

The exclusion of defective folate metabolism as the cause for the high tHcy concentrations in hemodialysis patients provides further support to our recent hypothesis that these high levels of homocysteine are due to a reduced clearance of this metabolite [15]. This reduction may be attributable to defective renal clearance and/or extrarenal clearance and metabolism. Impairment of homocysteine metabolism in hemodialysis patients [16] cannot provide a plausible explanation for the high homocysteine levels in these patients.

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REFERENCES

1. Bostom AG, Culleton B: Hyperhomocysteinemia in chronic renal disease: Disease of the month review. *J Am Soc Nephrol* 10:891–900, 1999
2. Bostom AG, Shemin D, Lapane KL, *et al*: High dose-B-vitamin treatment of hyperhomocysteinemia in dialysis patients. *Kidney Int* 49:147–152, 1996
3. Touam M, Zingraff J, Jungers P, *et al*: Effective correction of hyperhomocysteinemia in hemodialysis patients by folinic acid and pyridoxine therapy. *Kidney Int* 56:2292–2296, 1999
4. Bostom AG, Shemin D, Bagley P, *et al*: Controlled comparison of 5-L-methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. *Circulation* 101:2829–2832, 2000
5. Yango A, Shemin D, Hsu N, *et al*: L-folinic acid versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. *Kidney Int* 59:324–327, 2001
6. Bagley PJ, Selhub J: Analysis of folate form distribution by affinity followed by reversed-phase chromatography with electrical detection. *Clin Chem* 46:404–411, 2000
7. Said HM, Vaziri ND, Kariger RK, *et al*: Intestinal absorption of 5-methyltetrahydrofolate in experimental uremia. *Acta Vitaminol Enzymol* 6:339–346, 1984
8. Retief FP, Heyns AP, Oosthuizen M, *et al*: Aspects of folate metabolism in renal failure. *Br J Hematol* 36:405–415, 1997
9. Jennrette JC, Goldman ID: Inhibition of the membrane transport of folates by anions retained in uremia. *J Clin Lab Med* 86:834–843, 1975
10. Livant EJ, Tamura T, Johnston KE, *et al*: Plasma folate conjugase activities and folate concentrations in patients receiving hemodialysis. *J Nutr Biochem* 5:504–508, 1994
11. Massy ZA: Reversal of hyperhomocysteinemia in chronic renal failure: Is folic or folinic acid the answer? *Nephrol Dial Transplant* 14:2810–2812, 1999
12. Selhub J, Miller JW: The pathogenesis of homocysteinemia: Interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *Am J Clin Nutr* 55:131–138, 1992
13. Tremblay R, Bonnardeaux A, Geadah D, *et al*: Hyperhomocysteinemia in hemodialysis patients: Effects of 12-month supplementation with hydrosoluble vitamins. *Kidney Int* 58:851–858, 2000
14. Schmitz JC, Stuart RK, Priest DG: Disposition of folic acid and its metabolites: A comparison with leucovorin. *Clin Pharmacol Ther* 55:501–508, 1994
15. Bostom AG, Brosnan JT, Hall B, *et al*: Net uptake of plasma homocysteine by the rat kidney in vivo. *Atherosclerossis* 116:59–62, 1995
16. Van Guldener C, Stehouwer CD: Homocysteine-lowering treatment: An overview. *Expert Opin Pharmacother* 2:1449–1460, 2001